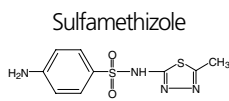
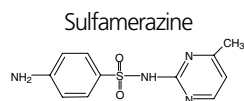
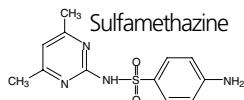
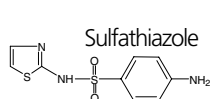


## Antibiotics (Sulfa Drugs) from Serum

These four antibacterial agents in the sulfa drug family can be extracted from serum. After acidifying a diluted serum sample, recoveries for the compounds are greater than 90%, using a simple methanol and water-based SPE method.



G000597, G000598,  
G000599, G000600

## Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Sulfathiazole	1.0µg/mL	90.1	±2.7
	5.0µg/mL	97.7	±2.1
2. Sulfamerazine	1.0µg/mL	918	±2.8
	5.0µg/mL	972	±2.4
3. Sulfamethazine	1.0µg/mL	919	±2.8
	5.0µg/mL	96.5	±2.2
4. Sulfamethizole	1.0µg/mL	88.7	±3.2
	5.0µg/mL	98.3	±2.5

## SPE

### SPE Procedure, Using Zymark RapidTrace SPE Workstation

Step	Solvent/ Solution	Volume (mL)	Flow Rate (mL/min)	Comments
1. Condition	MeOH	2.0	5.0	conditions sorbent
2. Condition	H <sub>2</sub> O	2.0	5.0	conditions sorbent
3. Load	spiked porcine serum	2.0 <sup>A</sup>	0.75	applies serum sample
4. Rinse	5% MeOH in H <sub>2</sub> O	2.0	5.0	washes sorbent
5. Purge-Cannula	H <sub>2</sub> O	4.0	30.0	clean sample cannula
6. Rinse	vent	0.1	2.0	positions SPE tube over waste port
7. Dry	N <sub>2</sub>	Time = 10 min		dries sorbent
8. Purge-Cannula	MeOH	4.0	30.0	cleans sample cannula
9. Collect	MeOH	1.0	1.0	elutes analytes into collection vessel
10. Collect	vent	6.0	3.0	pushes residual eluent into vessel <sup>B</sup>
11. Purge-Cannula	H <sub>2</sub> O	4.0	30.0	cleans sample cannula

<sup>A</sup> 1 mL porcine serum spiked 1.0µg/mL or 5.0µg/mL, diluted with 1 mL water, then acidified with 40µL H<sub>3</sub>PO<sub>4</sub>.

<sup>B</sup> Eluent evaporated to dryness with a nitrogen stream at 40°C, using a Zymark TurboVap LV Workstation, then reconstituted with 1 mL mobile phase.

## Antibiotics (Sulfa Drugs) from Serum

### HPLC Conditions:

Discovery C 18,  
15cm x 4.6mm, 5µm particles,  
preceded by 2cm C 18 guard  
column and 0.5µm frit filter.  
MeOH: 1% acetic acid (8:92)  
1.0mL/min (8 min) then  
0.6mL/min  
30°C  
UV, 254nm  
10µL reconstituted porcine  
serum extract

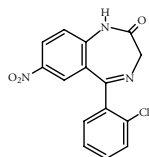
### SPE Tube:

Discovery DSC-18  
500mg/3mL

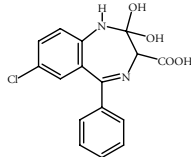
## Anticonvulsants

These drugs contain a benzodiazepine nucleus. Clonazepam is the most polar, due to the presence of the nitro substituent, and shows pK<sub>a</sub> values of 1.5 and 10.5. Clorazepate remains as the potassium salt under the neutral mobile phase conditions and is moderately acidic. Diazepam is the most hydrophobic of the three and is a weaker acid with a pK<sub>a</sub> of 3.4. Clonazepam and clorazepate contain an NH moiety capable of H-bonding with the amide group of the RP-AmideC16 stationary phase and hence is retained longer on this phase. Diazepam is retained longer on the C 18 phase, as expected from its hydrophobic interactions.

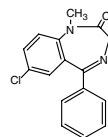
Clonazepam (IS)



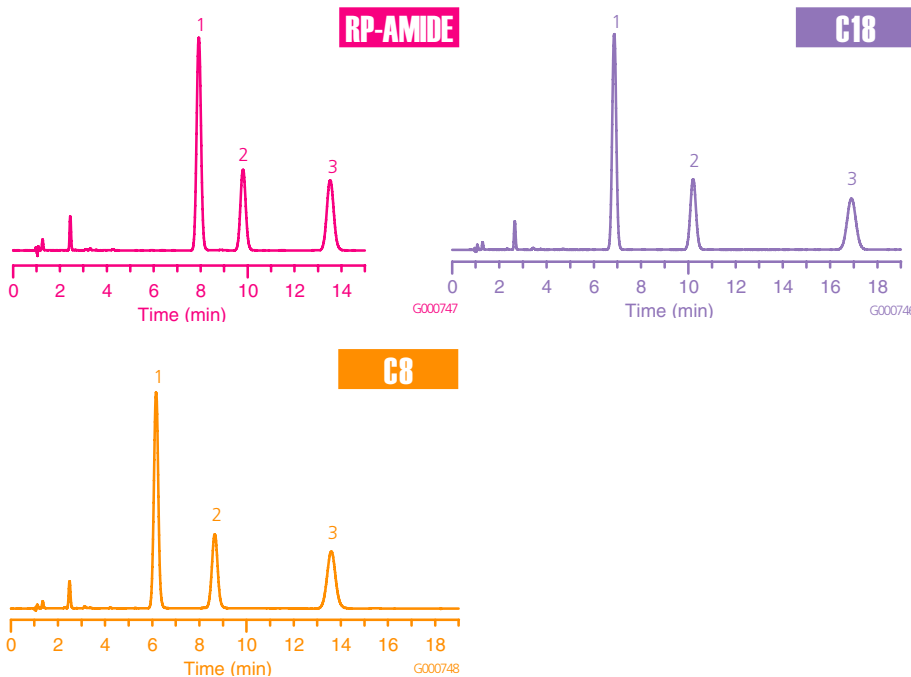
Clorazepate



Diazepam



G001211, G000199, G000200



## Anticonvulsants

15cm x 4.6mm columns,  
5µm particles,  
MeCN:H<sub>2</sub>O (30:70)  
2mL/min  
20°C  
UV, 254nm  
10µL

1. Clonazepam (IS)
2. Clorazepate
3. Diazepam